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Molecular Dynamics of Di-palmitoyl-phosphatidyl-choline Biomembranes in Ionic Solution: Adsorption of the Precursor Neurotransmitter Tryptophan

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Abstract

Microscopic structure of a fully hydrated di-palmytoil-phosphatidyl-choline lipid bilayer membrane in the liquid-crystalline phase has been analyzed with all-atom molecular dynamics simulations based on the recently parameterized CHARMM36 force field. Within the membrane, a single molecule of the α-aminoacid tryptophan (precursor of important neurotransmitters such as serotonin and melatonin) has been embedded and its structure and binding sites to water and lipids have been explored. In addition, properties such as radial distribution functions, hydrogen-bonding, energy and pressure profiles and the potentials of mean force of water-tryptophan and lipid-tryptophan have been evaluated. It has been observed that tryptophan usually has a tendency to place itself close to the lipid headgroups but that it can be fully hydrated during short time intervals of the order of a few nanoseconds. This would indicate that, for tryptophan, both hydrophobic forces as well as the attraction to polar sites of the lipids play a significant role in the definition of its structure and binding states.

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Keywords: Biomembranes, DPPC, tryptophan, neurotransmitter, ionic aqueous solution

1 Introduction

Biomembranes are ubiquitous in nature as limiting structures of cells, separating their contents from external environments, but allowing the exchange of nutrients and wastes through them. Mammalian cell membranes are complex structures protecting cellular contents, which include a wide variety of organelles (mithochondria, nucleus, lysosomes, vacuoles, Golgi apparatus, centrioles, ribosomes, etc.) surrounded by the cytoplasm. Transport of substances through cell membrane is of fundamental importance for human life. The principal components of human cellular membranes are lipids, cholesterol and proteins, all of them surrounded by aqueous solutions including water and salts. Phospholipid membranes provide the framework to biological

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membranes, to which other molecules (such as proteins or cholesterol) attach. They consist of two leaflets of amphiphilic lipids which self-assemble due to the hydrophobic effect[1]. Such lipids are molecules with a hydrophilic head and one or two lipophilic (and hydrophobic) tails. When placed inside aqueous ambient they can form three main different classes of structures: micelles, liposomes and bilayers, with the latest hiding the tails from water[2]. Since these molecules are not chemically bound between them, not only their local (vibrational) motions can be analyzed but also their long-range correlated motions (diffusion) can be measured and studied with all details with computer simulations. The main function of phospholipid as building blocks of cell membranes was already discovered in 1925[3].

In this paper we have focussed our efforts in two directions: on the one side, the study of phospholipid membranes can help understand basic biological membrane functions and its interaction with the environment. Among a wide variety of lipids, di-palmitoyl-phosphatidylcholine (DPPC) is one of most important, being a major constituent (about 40%) of pulmonary lungs[4]. A large number of simulations have already been performed on it, often including the influence of cholesterol in water environments[5]. On the other side, the role of proteins and drugs and their interactions with the membrane structure is undoubtedly a relevant field of research. To shed light on one hot topic, we have considered the introduction of a small biological probe into the lipid bilayer structure: the aminoacid tryptophan[6] (TRP), a precursor of the transmitters serotonin and melatonin[7]. Since the human body cannot synthesize such aminoacid and that it is needed to prevent diseases and death, it has to be acquired from the diet. Then, it is able to act as a building block in protein biosynthesis, while proteins are fundamentals required to sustain life. In addition, tryptophan functions as a biochemical precursor for the neurotransmitter serotonin[8]. Serotonin, in turn, can be converted to melatonin (a neurohormone), that may help humans to the regulation of biological rhythms, to induce sleep, to work as a strong antioxidant and also contribute to the protection of the organism from carcinogenesis and neurodegenerative disorders[9]. So, given the importance of tryptophan, we have explored its interactions with DPPC and water in a salty solution of sodium chloride as well as its local structure, molecular bonding and free energy profiles.

2 Computational details

Our system is a prototype model of an aqueous bilayer membrane composed by 204 lipids distributed in two leaflets of 102 flexible DPPC ($C_{40}H_{80}NO_8P$) molecules surrounded by 4962 TIP3P[10] water (W) molecules plus 17 sodium and 17 chloride ions, corresponding to physiological concentration and one tryptophan ($C_{11}H_{12}N_2O_2$) molecule. Sketches of the backbone structure of DPPC and of tryptophan are represented in Fig.1. Tryptophan and each DPPC molecule are described with atomic resolution (27 and 130 sites, respectively). Molecular dynamics (MD) simulations were performed with the NAMD2 simulation package[11] at a temperature of 310.15 K and an average pressure of 1 atm. The simulation time step was set to 2 fs. The recently parameterized force field CHARMM36[12], which is able to reproduce the area per lipid in excellent agreement with experimental data, has been used. All molecular bonds have been left non rigid, allowing fluctuations of bond distances and angles. Van der Waals interactions were cut off at 12 Å with a smooth switching function starting at 10 Å. Long ranged electrostatic forces were computed with the help of the particle mesh Ewald method[13], with a grid space of about 1 Å. Electrostatic interactions were updated every time step and periodic boundary conditions were applied in all three dimensions. After equilibration (for about 10 ns), two 25 ns production runs were generated, with a simulation box of size: 74.4Å× 74.4Å×

70.1Å . The temperature was controlled by a Langevin thermostat[14] with a damping coefficient of 1 ps^{-1}, whereas the pressure was controlled by a Nosé-Hoover Langevin barostat[15] with a damping time of 50 fs. Finally, in order to eliminate any artificial drift of the center-of-mass of the system in the simulations, the coordinates of lipid atoms were corrected for the motion of the center-of-mass of the monolayer they belong[16]. In a previous simulation where di-myristoil-phosphatidyl-choline (DMPC) lipid was modeled[17], a wide variety of properties of the simple lipid bilayer were satisfactorily reproduced (surface area per lipid, lateral pressure profile, order parameter of the lipid tails, etc.), so that we have not included these verifications here.

A general view of the system is shown in Fig.2. There, for the sake of clarity, water has been hidden so that only tryptophan, DPPC and sodium and chloride ions have been shown. We have chosen two significant configurations, one for TRP adsorbed to sites located at the headgroup in DPPC (left side of Fig.2) and another one for water solvating TRP (right side of Fig.2). We should point out that TRP tends to stay close to the headgroup regions of the lipid chains during most time of the simulated trajectories: An estimation over the full trajectory length indicated that around 70% of time TRP is attached to DPPC.

3 Results

3.1 Stability of the system

Before addressing the structural organization of the system, we checked the stability of the simulations and represented the contribution of all kinds of energies to the total energy of the system (Fig.3) as well as the temperature and the pressure (Fig.4), in order to ensure that our calculations were based on fully equilibrated MD runs.

On the one hand, the two main contributions to the total energy are the kinetic and the potential terms, as usual. In a deeper level of classification, potential energy has six contributions, namely those of: (a) molecular bonds; (b) molecular, dihedral and improper angular terms; (c) Van der Waals and (d) electrostatic (Coulomb) terms. Among all these terms, the electrostatic one is the largest by far, as expected. All contributions of the energy are shown in the last 10 ns of the simulation run, revealing stable profiles with fluctuations up to 3 % of the averaged values. On the other hand, we found some fluctuations smaller than 2 % for the temperature and of about 25 bar for the pressure. The mean pressure is zero, indicating that the bilayer is not affected by external or internal neat forces. From experimental and theoretical works, the expected surface tension of a lipid bilayer membrane should be zero[18, 19, 20, 21, 22] what it is consistent with the fact that the thermodynamic pressure of our system fluctuates around zero.

At the equilibration stage of the simulation, we placed TRP initially at the center of the membrane. After a few nanoseconds, we observed that TRP moved quickly to the water-DPPC interface and stayed there in close contact to the lipid headgroups. The most stable configurations of TRP at the interface and when solvated by water will be discussed below.

3.2 Structure of tryptophan around water and lipids

The local structure of the system can be analyzed by means of atomic radial distribution functions (RDF) $g_{AB}(r)$. For a species B close to a tagged species A, they are given by

$$g_{AB}(r) = \frac{V \langle n_B(r) \rangle}{4\, N_B\, \pi r^2\, \Delta r}, \tag{1}$$

where $n_B(r)$ is the number of atoms of species B surrounding a given atom of species A inside a spherical shell of width $\Delta r = 0.1\,\mathring{A}$. V stands for the total volume and N_B is the total number of particles of species B. The four $g(r)$ considered were defined for the pairs:

1. The hydrogen of tryptophan ('HT', corresponding to the three hydrogens labeled 'H1', 'H2' and 'H3', see Fig.1) *versus* the oxygen of a water molecule ('OW');

2. The oxygens of tryptophan ('OT', corresponding to the sites labeled 'O1' and 'O2', see Fig.1) *versus* the hydrogen of a water molecule ('HW');

3. The hydrogen of tryptophan 'HT' *versus* the negatively charged oxygen of DPPC ('O^-'), located at the lipid headgroups (label 'O2' in Fig.1);

4. The oxygens of tryptophan 'OT' *versus* the charged nitrogen of DPPC ('N^+'), located at the lipid's heads (see Fig.1).

The results are shown in Fig.5. The four radial distribution functions show some fluctuations in their profiles, the statistical noise observed in the association of TRP with N^+ of DPPC being more marked than for TRP-Water and for TRP-O^- of DPPC. We can observe a first coordination shell in all cases, being the binding of TRP to water the one with the highest peaks of the corresponding g(r)s (those depicted in the left column of Fig.5). In such a case, a second coordination shell can be also observed. Throughout our long simulation runs used to collect statistically meaningful properties, we observed periods of time of about 5 ns where TRP was fully solvated by water, indicating that the hydration of TRP is one stable state of the system, i.e. TRP can be fully hydrated for significant periods of time, essentially through hydrogen-bonding between the hydrogens of tryptophan (labeled as 'H1', 'H2' and 'H3' in Fig.1 and the water's oxygen. If we focus on TRP-DPPC binding, we can observe that the corresponding radial distribution functions $g_{HT-O^-}(r)$ and $g_{OT-N^+}(r)$ (depicted in the right column of Fig.5) show marked first coordination shells and very smooth second shells. This fact indicates that solvation of TRP by DPPC is carried out by a few lipid chains. From our simulation runs we observed that the average time of TRP inside the DPPC bilayer is of the order of 10-15 ns. The structure of water around DPPC is very similar to that of water around DMPC as it was described in a previous work[17] (Section III).

3.3 Hydrogen bonding of tryptophan with water and lipids

We can use a geometrical definition of a hydrogen bond, in the fashion as it is usually assumed in most computer simulations of water and associated liquids (see for instance a detailed study in Ref.[23]). It consists in considering that a hydrogen-bond is formed between two molecules when the next two geometrical conditions are fulfilled:

1. the distance R_{AH} between the "acceptor" molecule 'A' and the hydrogen "donor" atom 'H' is smaller than R^c_{AH},

2. the H-B$\cdots$A angle φ is lower than φ^c. Here 'B' is the atom to which 'H' is chemically bound.

In our study the threshold distances were taken from standard values in the literature. So, $R^c_{\mathrm{AH}} = 3.0$ Å for all types of HBs and the angular cutoff was chosen to be $\varphi^c = 30°$. This geometric definition has been found to be more adequate to describe intermolecular bonding in a wide variety of ambients, including supercritical environments, than alternative energetic criteria.[23].

With this HB definition, we computed the average number of HBs between several bound pairs. Our results indicate that we had (on average) a number of 3.2 water-water HBs per water molecule; around 1.9 HB for DPPC-water; about 2 HB between TRP and DPPC and ≈ 5 HBs for water-TRP. Furthermore, we can extract additional information from the radial distribution functions presented in Fig.5. There we can observe a sharp maximum located around 1.85 Å in three cases: HT-OW, OT-HW and HT-O^-. Since such distance is the signature of a typical oxygen-hydrogen HB, we can safely assume that HBs have been found between TRP and water as well as between TRP and the oxygen labeled 'O2' (see Fig.1). Given the simulation data collected, we did find clear HB-signature peaks for TRP with oxygens 'O6-O8' as well, but in a lesser extent than for oxygen 'O2', which is the preferred site for TRP-DPPC binding. This would indicate that TRP is also able to stay bound to the inner part of lipid heads during significant periods of time.

3.4 Potentials of mean force for tryptophan solvation

Once the local structure around TRP has been obtained, a common way to analyze the microscopic forces acting on it is by means of the so-called potential of mean force between species A and B, namely $PMF_{AB}(r)$, that can be readily obtained from the $g_{AB}(r)$ given in Eq.1:

$$PMF_{AB}(r) = -k_B T \ \ln g_{AB}(r), \tag{2}$$

where k_B is Boltzmann constant and T is the temperature.

The use of one-dimensional (and usually geometrical) reaction coordinates is simply an approximation to the real ones[24], which may be in general multidimensional, presumably involving a limited number of water molecules and, eventually coordinates or distances to the other species of the system. A method which does not assume any preconceived reaction coordinate is the so-called transition path sampling[25] a very specific computational tool requiring a huge amount of computational time. So, since the determination of the true reaction coordinate for the adsorption of tryptophan is out of the scope of this paper, we will consider radial distances between two species as order parameters useful to account for mean forces between them.

The results of our calculation are displayed in Fig.6. With the same order as in Fig.5, we show PMF for TRP-Water at the plots in left column and those for TRP-DPPC in plots at the right column of Fig.6. A free energy barrier is seen in all cases, defined by a neat first minimum and a second minimum less clearly defined, especially for TRP-DPPC interactions. In order to quantify the height of all barriers, we included a numerical estimation of them in Table 1.

Here we should note that in our system k_BT$\sim$10.78 kcal/mol, so that the physical values for the free energy barriers are of between 7.5 and 21.6 kcal/mol, i.e. the same order of magnitude of the free energies of adsorption of metal ions in DMPC membranes[26]. From the data reported in Table 1 we observe that the highest barrier corresponds to the pairing of TRP and water and that the most stable (averaged) distance for water closest to tryptophan is of about 1.7-1.8 Å , i.e. of the order of the typical HB distance, as pointed out above in Section 3.3. Conversely, the binding of TRP to DPPC happens with lower free energy barriers and at different distances between TRP and the binding site in DPPC. These data suggest that the configurational

Pair	ΔF (k_BT)	$\langle r_1 \rangle$ (Å)	$\langle r_2 \rangle$ (Å)
HT/O-W	2.0	1.8	3.2
OT/H-W	2.0	1.7	3.0
HT/O^--DPPC	1.0	1.8	3.3
OT/N^+-DPPC	0.7	4.0	$\approx$ 7.0

Table 1: Free energy barriers for the binding of tryptophan to water and DPPC. ΔF is the size (in k_BT) of the free energy barrier and $\langle r_{1(2)} \rangle$ are the position of the first (second) minima of PMF.

cost of the association of TRP to a lipid is lower than that of the association to water, what would explain why TRP tends to stay close to DPPC during periods of time about a factor 3 longer than the mean time remaining inside the water region of the system. To illustrate such association, we included a typical snapshot of this pair in Fig.7. Being this a preliminary study of TRP inside a model biomembrane, we have not computed yet the adsorption of ions to TRP sites. This is left for further analysis.

4 Concluding remarks

A series of molecular dynamics simulations of a DPPC lipid bilayer membrane in aqueous ionic solution of NaCl with an embedded single tryptophan molecule have been performed by MD using the CHARMM36 force field. The system has been stabilized for 10 ns and another two 25 ns runs have been employed to collect statistically meaningful properties.

We have focussed our analysis on the local structure of the tryptophan, when associated to water and to DPPC molecules. After this, the free energy of adsorption has been evaluated considering the usual one-dimensional reaction coordinates based on atomic distances for selected sites. We chose four types of particles: (1) the hydrogens labeled 'HT' and the double bonded oxygens 'OT' of tryptophan; (2) the three water sites; (3) the charged nitrogen labeled 'N^+' in DPPC and (4) the charged oxygen labeled 'O2' of DPPC. Our data revealed the existence of a strong first coordination shell and a milder second coordination shell for TRP-water structure, which translated to deep minima in the corresponding PMFs, with energy barriers for TRP-water association of the order of 10-20 kcal/mol. Conversely, the binding of tryptophan to DPPC involves a single coordination shell for the two sites of possible association (nitrogen and charged oxygen of DPPC *versus* the two tagged sites in TRP) and energy barriers much lower than for the former TRP-water, i.e. of the order of 7-10 kcal/mol.

Dynamics of tryptophan and lipids has not been evaluated, since it would have required simulation runs of the order of microseconds, out of the scope of this preliminary study. However, as a first indication of the dynamical aspects of the present system, we observed that TRP shows a clear tendency to stay close to DPPC, during periods of time about 3 times longer than for TRP-water association. As the topic for future MD studies, we plan to include different kinds of ionic species in the system and employ more sophisticated tools such as metadynamics to explore with more detail the free energy landscapes of TRP (plus other neurotransmitters such as serotonin and melatonin) in DPPC bilayer membranes, including cholesterol.

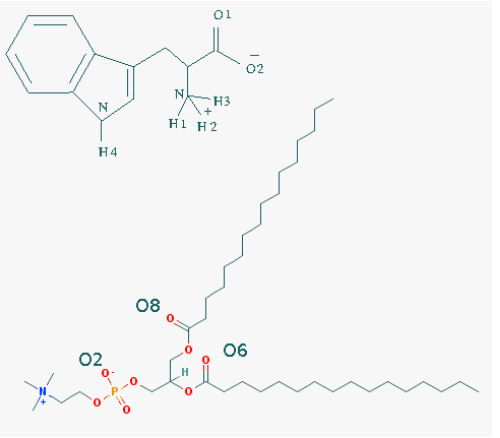

Figure 1: Sketches of the backbone structures of DPPC (bottom) and tryptophan (top). Hydrogens bound to carbon and nitrogen not shown. The highlighted sites are the most active ones and they will be referred in the text as indicated here.

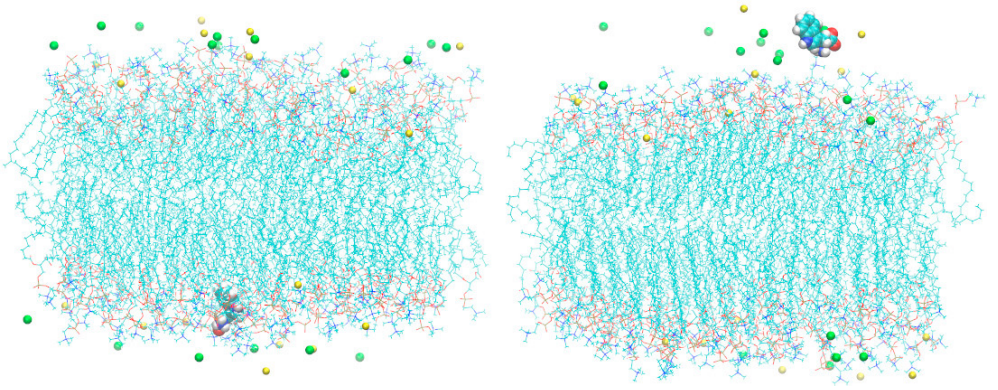

Figure 2: Two snapshots of the aqueous DPPC membrane with an embedded tryptophan molecule. Tryptophan inside DPPC (left) and inside water (right). Surrounding water is not shown for the sake of clarity. Atoms in TRP: Oxygen (red); hydrogen (white); carbon (cyan); nitrogen (blue). Sodium ions (yellow) and chloride ions (green).

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Figure 3: Energy decomposition for the full system.

Figure 4: Temperature and pressure fluctuations for the full system.

Figure 5: Selected radial distribution functions for tryptophan (T) with water(W) and DPPC (charged sites N and O2, see Fig.1): OT-HW (bottom left), HT-OW (top left), OT-N^+ (bottom right) and HT-O^- (top right).

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Figure 6: Potentials of mean force for water-tryptophan and DPPC-tryptophan sites. Labels as in Fig.5.

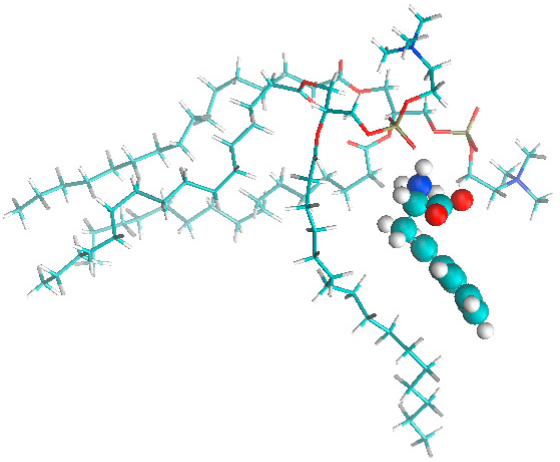

Figure 7: Snapshot of a typical tryptophan-DPPC bond. Atoms in TRP colored as in Fig.2.

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